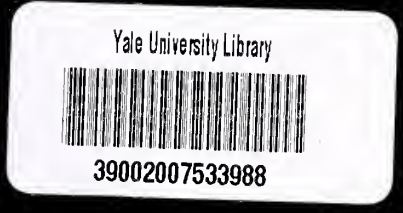
T113
T113
+Y12
2549



STUDIES ON THE PYROGENICITY OF PNEUMOCOCCUS IN NORMAL AND SENSITIZED RABBITS

THOMAS A. SAVIGNANO

1963

MUDD
LIBRARY
Medical

YALE

MEDICAL LIBRARY

Digitized by the Internet Archive
in 2017 with funding from
Arcadia Fund

https://archive.org/details/studiesonpyrogen00savi

STUDIES ON THE PYROGENICITY OF PNEUMOCOCCUS IN NORMAL AND SENSITIZED RABBITS

Thomas Augustine Savignano

A Thesis

Presented to

The Faculty of the Yale University School of Medicine

in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Medicine

Department of Internal Medicine

School of Medicine

Yale University

1963

YALE MEDICAL
JUN 1963
LIBRARY
T113
Y12
2549

To Dr. Atkins

who planted the idea

and to Janet

who tended its growth

the harvester

expresses his deepest gratitude

TABLE OF CONTENTS

INTRODUCTION 1

MATERIALS AND METHODS 12

RESULTS 21

DISCUSSION 35

SUMMARY 41

BIBLIOGRAPHY 43

STUDIES ON THE PYROGENICITY OF PNEUMOCOCCUS IN NORMAL AND SENSITIZED RABBITS

INTRODUCTION

The pathogenesis of fever in gram-positive infections is poorly understood. Several early reports have suggested that fevers following the injection of gram-positive organisms and those induced by either gram-negative bacteria or their endotoxins differ in their latent periods. More recently, studies in cross-tolerance between the two responses have confirmed the impression that the pyrogen of gram-positive organisms differs considerably from bacterial endotoxin (2).

An important contribution to the understanding of gram-positive pyrexia has come with the discovery of the role played by circulating pyrogens of endogenous origin in a wide variety of experimental fevers (3, 4, 18). In experiments involving the intravenous injection of various gram-positive cocci into rabbits it has been shown that the febrile response to these organisms is regularly associated with the release of endogenous pyrogen (2), probably from circulating leucocytes (1). This release is thought to be related to phagocytosis, since fevers with most gram-positive organisms are seen only after the injection of whole organisms and closely resemble

the fevers elicited by many inert particulate materials (6). On the other hand, recent studies with animals sensitized to various heterologous proteins (7, 11, 26) have provided evidence that fever may represent an allergic reaction to specific antigen. The possibility must therefore be considered that an element of hypersensitivity to gram-positive bacteria may function alongside mechanisms of cellular defense to produce fever.

The present paper is a study of the factors which induce fever in rabbits made hypersensitive to derivatives of pneumococcus. An effort is made to correlate the characteristics of this system with findings in other systems of hypersensitivity. The responses of normal rabbits to a variety of pneumococcal cell preparations have also been studied since controversy exists whether pneumococcus can produce fever in the absence of infection (5, 18).

Experimental Pneumococcal Fever

Two types of fever are commonly associated with the intravenous injection of bacteria into experimental animals. The first type of response is caused by gram-negative bacteria and by the endotoxins isolated from these organisms. It begins 15 to 30 minutes after injection, reaches maximum in 1 to 3 hours, and is characteristically biphasic. The second type of response, caused by gram-positive organisms, has a latency of 1 to 2 hours, reaches maximum

at 3 to 5 hours, and with sufficient dosage is also characteristically biphasic.

Certain workers who have injected live pneumococci of different types into the blood stream of rabbits and other laboratory animals have observed a febrile response which, like fevers caused by other gram-positive organisms, begins between 1 and 2 hours after injection (28). In comparative studies with different numbers of organisms the magnitude of this response has been found to be directly related to the numbers of bacteria injected, while the latency remains the same (1).

Important exceptions to reports of the 1 to 2 hour latency of pneumococcal fever are communications by Bennett and Goodner. Goodner (9), studying intradermal pneumococcal infection in rabbits, reported that body temperature did not rise until 5 to 12 hours after injection and that its onset appeared to be independent of the bacteremia since in several animals fever developed considerably before, and in several long after, organisms were detectable in the blood. Bennett (5) observed that the intravenous injection of $3 - 8 \times 10^9$ viable pneumococci Type I into normal rabbits produced no immediate febrile response although these animals later developed active infections with fever, the onset of the fever generally being 6 to 8 hours after injection. These

late fevers could be prevented by prior injection of the animals with penicillin.

There are also conflicting reports concerning the pyrogenicity of heat-killed pneumococci. In early experiments with pneumococcus, staphylococcus and other gram-positive bacteria, Hort and Penfold found that none of these organisms elicited fever when they were heat-killed at 100° C (14). Later workers have not corroborated this finding. Probey and Pittman (23) did not study pneumococci but found that the thermogenicity of staphylococcus is altered but not abolished by killing with heat. The fevers of both live and killed organisms were reported to be of one hour latency, but with dead organisms the temperature rose more quickly, reaching its peak at 3 to 5 hours and then falling. With live bacteria the temperature was still rising at 5 to 6 hours. Atkins and Freedman also observed that autoclaved pneumococci and staphylococci cause fevers one hour after injection (2).

The present paper describes the responses of normal rabbits to live, heat-killed and autolysed pneumococci given intravenously.

Pneumococcal Hypersensitivity

The antigenic structure of the pneumococcus has been

carefully studied (27). The chief components of the pneumococcal cell are the type-specific capsular carbohydrate (SSS) and species-specific somatic protein (nucleoprotein, NP). The latter is itself a combination of many antigens. Pneumococcal infection in animals and man gives rise to the type-specific anti-SSS antibody and to anti-proteins which are species-specific. Anti-SSS agglutinates only pneumococcal cells of the homologous type and precipitates the capsular carbohydrate of that type. Anti-NP agglutinates unencapsulated (R) pneumococcal cells and precipitates somatic proteins of all pneumococci. It also cross-reacts with certain strains of Streptococcus viridans. (21)

Julianelle observed that rabbits injected with heat-killed encapsulated (S) pneumococci by the intravenous route develop both anti-SSS and anti-NP titers, but develop only anti_NP when given the same cells intradermally (16).

Harley (12, 13) distinguished three types of dermal hypersensitivity in rabbits injected with pneumococci:

(1) Sensitivity to SSS. In this type of sensitivity, a positive skin reaction, consisting of erythema and edema, appears 3 to 6 hours after the skin test is performed, is maximal in 24 hours and disappears quickly. It occurs only in rabbits that have been injected with S cells intravenously. It is type-specific, is related to the level

of type-specific antibody, and can be passively transferred to normal rabbits by transfusion of serum containing anti-SSS.

(2) Sensitivity to NP. In this type, the skin reaction appears in 3 to 6 hours, is maximal in 24 hours and disappears in 3 to 4 days. It occurs in rabbits that have been injected either intravenously or intradermally with S or with R cells. It is species-specific, is related to the level of species-specific antibody, and can be passively transferred to normal rabbits by transfusion of serum containing anti-NP.

(3) Sensitivity to heat-killed whole cell vaccines. In this type, the skin reaction does not appear before 24 hours after testing and disappears very slowly. It occurs in rabbits that have been injected intradermally with S or with R cells. It is species-specific but is not related to titers of either anti-SSS or anti-NP. The reactive factor in the vaccine is thought to be the somatic protein.

Julianelle (17) sensitized rabbits with repeated intradermal injections of whole heat-killed S pneumococci and made a careful study of the relationship of skin reactivity to circulating anti-NP (measured as R-agglutinin). He found that his regimen of injections induced a type of delayed dermal reactivity to the heat-killed cells which was not related to the concentration of antibody in the blood in that it developed approximately 10 days before the appearance of

antibody, and became maximal when the concentration of antibody was still low. The animals also demonstrated a delayed type of reactivity when solutions of NP were instilled into the conjunctival sac and when they were injected into the skin. Normal rabbits transfused with antisera from the sensitized animals failed to develop conjunctival reactivity to NP but did demonstrate positive skin tests to NP. Unfortunately the transfused rabbits were not tested for sensitivity to the whole heat-killed cells. Julianelle concluded from this experiment that the intradermal injection of heat-killed S pneumococci into rabbits induces two types of sensitivity to the protein component of pneumococcal cells:

(1) An immediate Arthus-type sensitivity dependent on the presence of circulating antibody, and

(2) A delayed type of sensitivity independent of circulating antibody. These types are not easily distinguished by the latency of the skin test since the Arthus reaction may take 24 hours to reach maximum. Distinction is made primarily by the association with serum antibody.

Hypersensitivity Fever

Fever has long been recognised clinically as a cardinal manifestation of hypersensitivity, but only recently have the mechanisms

involved in its pathogenesis been studied. Fever regularly occurs in serum sickness in man and has been described in rabbits sensitized with heterologous sera (10). Farr (7) has reported that the majority of rabbits immunized by multiple intravenous injections of bovine serum albumin (BSA) respond to the specific antigen several weeks later with biphasic febrile responses resembling endotoxin fever. Daily injections of antigen resulted in a rapid tolerance which was lost after a short rest period. No cross-tolerance to endotoxins was demonstrable. Although in this experiment there was noted to be poor correlation between quantitative precipitin antibody titers and febrile response, antibody was present in all sensitive rabbits. Further studies have shown that sensitivity to BSA-induced fever can be transferred with serum but not with mononuclear cells from spleens of hypersensitive donor rabbits (8). This finding would appear to confirm the importance of circulating rather than cellular antibody in this system. It is postulated that intravascular combination of antigen with antibody may produce damage to circulating leucocytes with subsequent release of endogenous pyrogen (1).

Uhr and Pappenheimer (26) have reported fevers in guinea pigs that were sensitized to a variety of protein antigens and challenged intraperitoneally ten days later with a single dose of specific antigen.

Both the skin reactivity, which was of delayed type, and the febrile response were absent to repeated challenge on the second day. In later studies (25) there appeared to be no dosage of passively transferred circulating antibody which would sensitize normal animals to react to specific antigen with fever. This finding suggested that the febrile response in this system was dependent on cellular, as opposed to circulating antibody.

In studies on the mechanism of tuberculin-induced fever, Stetson (24) observed that rabbits sensitized with BCG responded to intravenously administered Old Tuberculin (OT) with high biphasic fevers similar to endotoxin fever. Hall and Atkins (11) made a careful investigation of this phenomenon and concluded from their findings that the febrile response of BCG-sensitized rabbits is a specific property of this system. The response to OT differed from that obtained with endotoxin in that there was a longer latent period of approximately one hour before onset of fever. Moreover, animals rendered tolerant to endotoxin showed no significant diminution in their response to OT. It was further observed that daily injections of OT in sensitized rabbits resulted in a rapid tolerance to its pyrogenic effect. Tuberculin tolerance could be differentiated from that occurring with endotoxins and was invariably associated with the

development of a negative skin test. The mechanism of this tolerance was inferred to be specific desensitization.

In other studies by Johanovsky it was found that rabbits passively sensitized by leucocytes of tuberculin-sensitive donors liberate endogenous pyrogen when challenged with PPD (15). Moses and Atkins made the additional observation that sensitivity to the pyrogenic effects of OT could not be transferred with serum from BCG-infected donors (unpublished observations). Both these findings support the suggestion of Hall and Atkins that febrile response in the tuberculin system is a manifestation of delayed, i.e. cellular, hypersensitivity.

Further studies along these lines (to be published) have been pursued by Atkins with rabbits environmentally exposed to certain strains of Staphylococcus aureus. He has observed that the intravenous injection into such rabbits of staphylococcal lysates and culture filtrates elicits a febrile response similar to that elicited by OT in tuberculin-sensitive rabbits, while animals that have been protected from exposure to the organism are non-reactive to the same inocula. This observation is offered as another example of fever due to hypersensitivity.

The present paper reports observations on the febrile and dermal responses of rabbits sensitized to autolysates of pneumococcus.

Experiments in cross-reactivity between pneumococcus and staphylococcus have also been carried out. An effort is made to correlate the results of these studies with findings in other systems of hypersensitivity.

MATERIALS AND METHODS

General

Male and female rabbits of several breeds, weighing 6 to 9 kg., were used in all experiments. They were housed in an air-conditioned room. Experiments were conducted in an adjacent room maintained at 65° to 70° F.

All glassware, filters, and needles were sterilized by dry heat at 170° C. for 2 hours to inactivate any contaminating pyrogens.

Water was prepared pyrogen-free by double distillation and autoclaving at 120° C. for 2 hours. Pyrogen-free water was used in all preparations of physiologic saline, culture media, and solutions of bile, NaOH, acetic acid, and trichloroacetic acid.

Temperature Recording

Rabbits were placed in aluminum boxes with openings for the head and rectum. To ensure a consistent response, recipients used for the first time were kept in these boxes for 5-hour periods on two days prior to the experiment.

On the day of an experiment, temperature readings were taken at 15 minute intervals for 1 1/2 hours to establish a baseline prior to injection. After injection into the marginal ear vein, temperatures were recorded every 15 minutes for 5 hours.

Temperatures were measured by rectal leads connected to the Foxboro rabbit scanning switch and fever recorder. Animals showing a variation of over 0.3° C. in the hour preceding injection or an initial temperature higher than 40° C. were not used.

Source and Preparation of Stock Cultures of Pneumococcus Type I

A culture of pneumococcus type I strain A-5 in defibrinated rabbit blood was obtained through the kindness of Dr. Barry Wood of the Johns Hopkins Department of Microbiology. This culture was used to inoculate 5 ml. normal rabbit serum. The serum was incubated at 37° C. for 18 hours. One drop of the 18 hour serum culture was added to each of several tubes containing 3 ml. defibrinated rabbit blood. After 18 hours of incubation each blood culture was streaked to a blood agar plate to determine if any contamination had occurred. The cultures were then covered with a layer of sterile melted vaseline and stored at 4° C. Cultures that proved to be contaminated were subsequently discarded.

Cell Counts

Pneumococcal cultures were counted by the serial dilution agar pour plate technique.

Preparation of Pneumococcal Autolysate

Pneumococci were routinely grown in tryptose phosphate broth (Difco) enriched with 1% defibrinated rabbit blood, 18 hour broth cultures (10^6 cells per ml.) were centrifuged and the cell button washed twice with pyrogen-free saline (PFS). The cells were finally resuspended in a volume of PFS equal to 1/50 the volume of the broth culture. To this suspension was added 1 ml. 10% Na desoxycholate solution. The suspension was let stand overnight to ensure complete lysis of cells. The bile lysate was centrifuged and the supernate filtered through a Seitz #6 filter. The filtrate was allowed to dialyze for 3 days against 50 volumes of PFS at 4° C. to remove the bile. The dialysate was brought to pH 8 with 1 N NaOH and was filtered through a Morton glass bacterial filter to remove particulate material. Two ml. of the filtered autolysate (equivalent to approximately 10^8 cells) were routinely tested in normal rabbits to rule out the presence of contaminating pyrogens. The autolysate was stored at 4° C. and used within two weeks. Just prior to use it was filtered again to remove the minute amounts of precipitate that occasionally formed.

In one experiment requiring the use of an autolysate of 3×10^9 cells, pneumococci were grown in normal rabbit serum. An 18 hour serum culture (10^9 cells per ml.) was centrifuged and the cell button washed twice with PFS. The cells were then resuspended

in a volume of PFS equal to 1/3 the volume of the serum culture. The remainder of the process is as described above.

Preparation of Heat-killed Pneumococcal Cells

Eighteen hour tryptose phosphage broth cultures of pneumococci (10^6 cells per ml.) were centrifuged and the cell button washed twice with PFS. The cells were finally resuspended in a volume of PFS equal to 1/3 the volume of the broth culture. The suspension was placed in a 56° C. water bath for one hour. It was then streaked to a blood agar plate to ascertain its sterility.

In one experiment requiring the use of 3×10^9 heat-killed cells, pneumococci were grown in normal rabbit serum. An 18 hour serum culture (10^9 cells per ml.) was centrifuged and the cell button handled as above.

Preparation of Pneumococcal Serum Filtrates.

Eighteen hour cultures of pneumococci in normal rabbit serum were filtered through a Morton glass bacteria filter to remove the cells. The filtrate was stored at 4° C.

Isolation of Nucleoprotein from Pneumococcal Autolysate

(A) Precipitation with TCA: A 50 per cent solution of trichloroacetic acid was made up with pyrogen-free water. This

solution was combined with a volume of pneumococcal autolysate so that the final concentration of acid was 11 per cent. After storage at 4° C. for 18 hours, the preparation was centrifuged and the supernate decanted and set aside. The protein precipitate was washed with PFS and resuspended in a volume of PFS equal to the original volume of the autolysate. The suspension was brought to pH 8 with 1 N NaOH in order to bring all the protein into solution. The solution was filtered through a Morton filter and stored at 4° C. until required for injection.

The TCA supernate was allowed to dialyze for 18 hours against 25 volumes of PFS at 4° C. to remove the TCA. The dialysate was neutralized with 1 N NaOH and passed through a Morton filter. It was then stored at 4° C. until used.

When subjected to quantitative protein determination, TCA supernates were found to be protein-free.

(B) Precipitation with Acetic Acid: A volume of pneumococcal autolysate was brought to pH 8 with 1 N NaOH in order to bring all the protein into solution. This preparation was centrifuged to remove non-specific debris and the supernate passed through a Morton filter. To the filtrate 1 N acetic acid was added dropwise until flocculation occurred. This suspension was centrifuged and the supernate decanted and set aside. The protein precipitate

was washed in PFS and resuspended in a volume of PFS equal to the original volume of the autolysate. The suspension was brought to pH 8 with 1 N NaOH and passed through a Morton filter. The filtrate was stored at 4° C.

The acetic acid supernate was allowed to dialyze for 18 hours against 25 volumes of PFS at 4° C. to effect the removal of the acetic acid. The dialysate was then stored at 4° C.

When quantitative protein determinations were performed, acetic acid supernates were found to retain small quantities of protein.

Quantitative Protein Determinations

Solutions of pneumococcal nucleoprotein and the supernates of TCA and acetic acid preparations were subjected to quantitative protein determination prior to their experimental use. Determinations were performed by the Folin-Ciocalteau method (19). Readings were made on a Klett photometer.

Stock Culture of Staphylococcus

A culture of Staphylococcus aureus type Giorgio on a blood agar plate was obtained from Dr. Leanor Haley of the Yale Microbiology Department. This was stored at 4° C. and was periodically subcultured by inoculating beef heart infusion broth (Difco) and streaking the 18 hour broth culture onto a blood agar plate.

Preparation of "Mickled" Staphylococcal Filtrate

Eighteen hour cultures of staphylococcus Giorgio in beef heart infusion broth were centrifuged and the cell button washed twice with PFS. The cells were finally resuspended in a volume of PFS equal to 1/4 the volume of the blood culture. The resuspended cells were disrupted by agitation with 5 grams of #100 glass beads in a Mickle apparatus for 45 minutes. The mickled preparation was filtered through a Morton filter to remove particulate materials. Four ml. of the filtrate was routinely tested in normal rabbits to rule out the presence of contaminating pyrogens. A febrile response within 30 minutes of injection was considered evidence of endotoxin contamination. The filtrate was stored at 4^{o} C. and used within two weeks.

Vaccination of Animals*

Freund's complete adjuvant and a saline suspension of heat-killed pneumococcal cells (3×10^{6} cells per ml.) were combined in

* Preliminary attempts at sensitization included the intravenous and the intradermal injection of 10^{3} - 10^{7} live pneumococci. All animals in this group died within 48 hours of injection despite treatment with penicillin. Other attempts to sensitize included series of daily intravenous injections of heat-killed and of autolysed pneumococci interrupted with one- week rest periods. High mortality, presumably from shock, was also noted in this group. Of the survivors few developed a positive skin test. Because of the poor results, these methods of sensitization were discarded.

equal volumes in a glass homogenizer. 0.2 ml. of the homogenate was injected intradermally into the fore toepad of each recipient rabbit. After three days a second injection was made into the toepad of the other forefoot. Animals were skin tested 10 days after the second injection. Tests for febrile reactivity were performed 2 to 3 weeks after the second injection.

Skin Tests

Skin tests were performed by the intradermal injection of 0.2 ml. of pneumococcal autolysate into a depilated abdominal site. Testing was done 10 days after vaccination with heat-killed cells in Freund's adjuvant. Normal rabbits were used as controls.

Eighteen hours after the test injection, about 88 per cent of the vaccinated recipients developed a round circumscribed area of erythema at the injection site with marked central edema. The lesions were read at 24 hours and recorded as "millimeters of erythema". They persisted until 48 hours and then quickly disappeared.

Skin reactivity was noted in none of 20 normal controls.

Test for Serum Precipitins

The marginal ear vein of normal and vaccinated rabbits was nicked with a razor and samples of blood collected and centrifuged. The undiluted sera were tested against undiluted pneumococcal

autolysate by means of the two basin plate technique of Ouchterlony (22). Plates were examined daily for a week for the development of bands of precipitate between the two wells.

Fever Charts

Fever curves were plotted on 1/6 inch graph paper, with five lines on the ordinate representing 1^{o} C. and six lines on the abscissa for each hour. In order to obtain a numerical expression of both the height and duration of fever, an arbitrary "fever index" was calculated by dividing by 9 the number of squares beneath each 5 hour fever curve. In order to define the area a perpendicular line was drawn from the 5 hour temperature to the baseline.

RESULTS

Pyrogenicity of Pneumococcal Preparations in Normal Rabbits

The ability of live and heat-killed pneumococci to elicit fevers when injected intravenously into normal rabbits has been the subject of conflicting reports. While Bennett noted that live pneumococci do not induce fevers until 6 to 8 hours after injection (5), and Hort and Penfold observed that the heat-killed organisms are not pyrogenic (14), other observers have reported the development of fevers 1 to 2 hours after the injection of viable (28) and of heat-killed pneumococci (1).

It was decided to initiate the present study by giving normal rabbits intravenous injections of 3×10^9 pneumococci type I in the live, heat-killed and autolysed state. Live and heat-killed cells were administered in saline suspension. All inocula were one ml. in volume. The results are shown in Fig. 1.

Noteworthy are: (a) the 90 minute latency of fevers elicited by live and by heat-killed cells; (b) the biphasic character of the response to live cells; (c) the more gradual rise and rapid fall of fever induced by heat-killed cells; and (d) the failure of autolysed cells to induce fever.

These findings indicate the following points:

(1) In latency and character of response fevers induced by intravenously administered pneumococci do not differ from those induced by other gram-positive organisms;

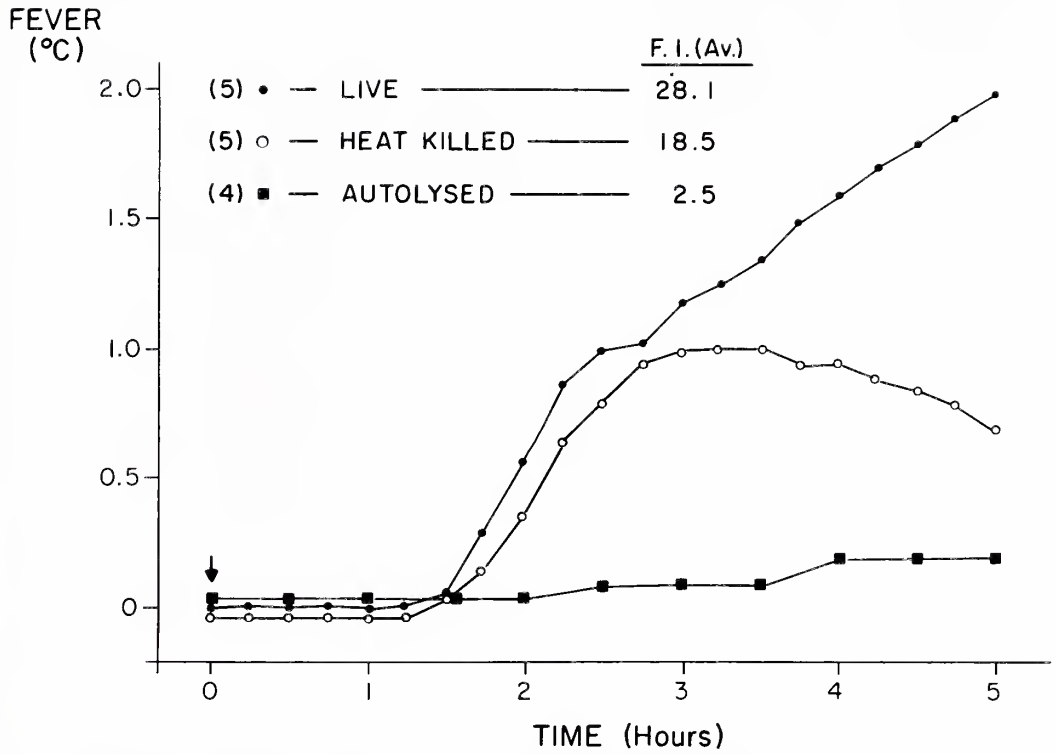


Fig. 1 Mean febrile responses of normal recipients to 3×10^9 pneumococcal cells in the live, heat-killed and autolysed state. Figures to the left of key represent number of animals in each group, those to the right indicate the mean fever indices (F. I.) of each group.

(2) the pyrogenicity of the intact cell is somewhat diminished, but not abolished, by killing with heat;

(3) in normal animals the integrity of the cell body is essential to its pyrogenicity.

Pyrogenicity of Pneumococcal Autolysate in Rabbits Vaccinated with Heat-Killed Pneumococci

Forty-one rabbits vaccinated with heat-killed pneumococci in Freund's adjuvant (see Methods) were given an intravenous injection of 2 ml. of pneumococcal autolysate two to three weeks after vaccination. Eight normal rabbits were injected as controls. Of the 41 vaccinated animals, 28 developed fevers greater than 0.5° C. when injected with autolysate. In none of the control group was febrile reactivity observed. The results are shown in Fig. 2.

The following consistent characteristics of the febrile reaction to autolysate are worthy of note: (a) a latent period of 30 to 60 minutes; (b) a biphasic response with a maximum temperature elevation of 1.0° to 1.7° C. reached in four to five hours.

Fevers of similar type have been described in BCG-infected rabbits after injection with OT (11). As in the tuberculin system, the eliciting material is not pyrogenic in normal animals.

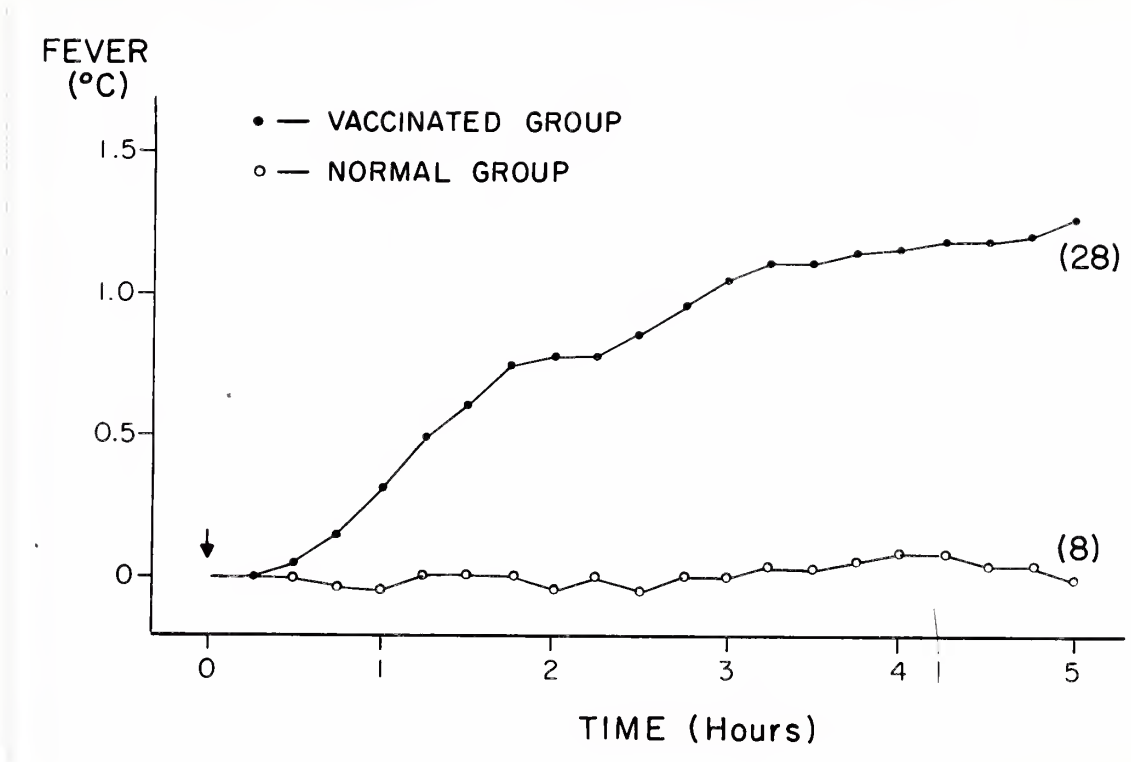


Fig. 2 Mean febrile response of 28 vaccinated recipients to 2 ml. pneumococcal autolysate. Average response of eight normal recipients is shown for comparison.

Relation of Skin Reactivity to Febrile Response in Vaccinated Rabbits

The lack of correlation between the magnitude of the skin reaction and of the febrile response to autolysate in 41 vaccinated rabbits tested two weeks after vaccination is illustrated in Fig. 3. Note also that of the 28 animals with fevers greater than 0.5° C. (fever index 6), two animals did not demonstrate skin reactivity; conversely, of the 13 rabbits with fevers less than 0.5° C., 10 rabbits had positive skin tests.

It appears that dermal sensitivity is not always accompanied by febrile reactivity, though the great majority of significantly febrile animals are skin-sensitive.

Relation of Skin Reactivity to Presence of Circulating Antibody

Julianelle (17) observed that rabbits given intradermal vaccinations of heat-killed pneumococci develop two types of dermal reactivity to pneumococcal protein: (a) an immediate Arthus-type reaction dependent on the presence of circulating precipitating antibody, and (b) a delayed reaction independent of circulating antibody.

It was considered advisable to determine which type of skin reaction was the one produced in the present study. Accordingly, 14 vaccinated and four normal rabbits were tested for skin reactivity to autolysate. Several days after skin testing, serum samples were

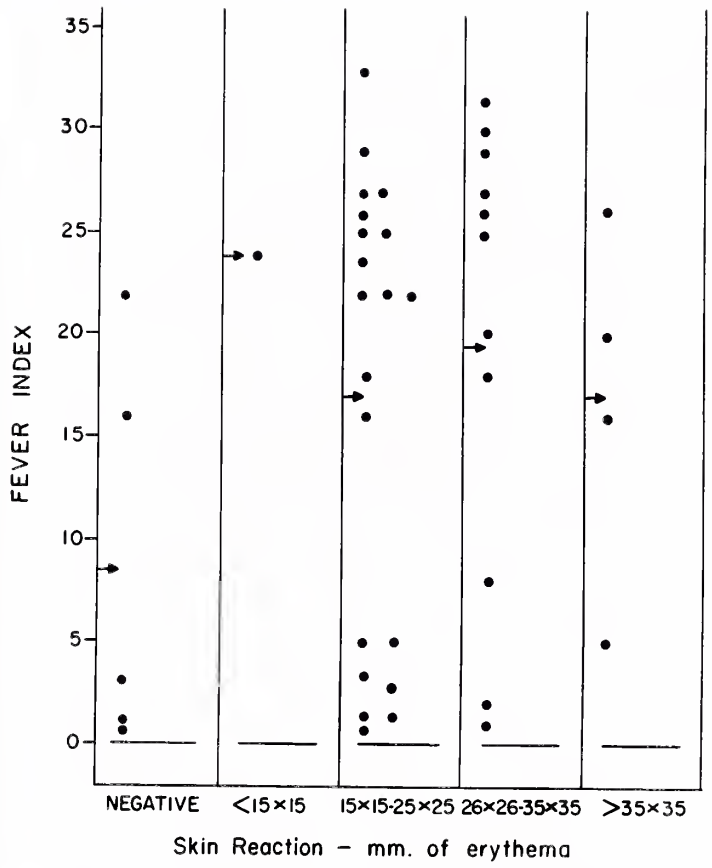


Fig. 3 Correlation of skin reactivity with fevers induced by 2 ml. autolysate in 41 vaccinated animals. Arrows indicate the mean fever index for each group. "Negative" animals were vaccinated but had negative skin reactions.

collected and tested against autolysate for the presence of precipitating antibodies (see Methods). Results are shown in Fig. 4.

It is seen that of 13 animals with positive skin reactions, six had detectable serum precipitins, while seven did not. Under these conditions skin reactivity does not appear to be dependent on the presence of precipitating antibodies in the blood and would seem, therefore, to be a manifestation of delayed, rather than immediate, hypersensitivity.

Relation of Febrile Response to Presence of Circulating Antibody

Shortly after the collection of sera for antibody studies, all the vaccinated and normal rabbits employed in the above experiment were given intravenous injections of 2 ml. of autolysate. The vaccinated animals that proved to have detectable serum precipitins at this time were tested again ten weeks later for serum precipitins and for febrile reactivity to autolysate. The results are summarized in Table I.

Note that two weeks after vaccination, at the height of febrile response to autolysate, serum precipitins were absent in eight of the 14 animals tested. This suggests that the presence of circulating precipitin is not a prerequisite for fever. On the other hand, two animals with markedly reduced febrile responses to autolysate at

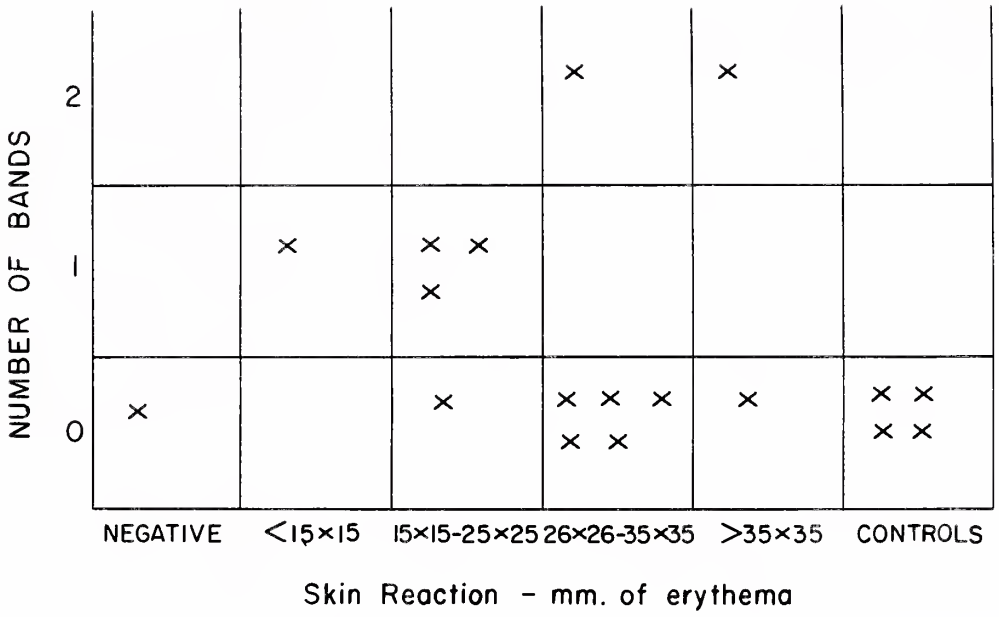


Fig. 4 Correlation of skin reactivity and presence of serum precipitins in vaccinated rabbits. The Ouchterlony two basin plate technique was used for the antibody studies. All four control animals were skin test negative.

TABLE I

CORRELATION OF FEBRILE REACTIVITY AND PRESENCE OF SERUM PRECIPITINS IN NORMAL AND VACCINATED RABBITS

	Number in group	Bands observed		Mean fever index
Normal animals	4	no bands	4	0
		one band	--	--
		two bands	--	--
Animals vaccinated two weeks previously	14	no bands	8	22
		one band	4	26
		two bands	2	29
Vaccinated animals with bands at two weeks re-tested at 12 weeks.	6	no bands	4	4
		one band	1	4
		two bands	1	5

two weeks nevertheless retained their serum antibodies to pneumococcus, indicating that precipitins may be present when febrile responses to autolysate are diminished or absent.

Pyrogenicity of Pneumococcal Nucleoprotein in Rabbits Reactive to Autolysate

With evidence that skin reactivity of vaccinated animals is a manifestation of delayed hypersensitivity to pneumococcal protein, it was decided to test for the pyrogenicity of isolated pneumococcal protein in animals reactive to autolysate.

Accordingly three vaccinated animals were injected on separate occasions with the following materials:

(a) 2 ml. autolysate

(b) nucleoprotein derived from the same autolysate preparation by precipitation with TCA and resuspended in saline to the original volume of autolysate. Two ml. were injected.

(c) the supernate remaining after protein precipitation. Since this solution was found to be protein-free by quantitative determination it was inferred to contain the pneumococcal carbohydrate fraction. Four ml. were injected. The results are shown in Fig. 5.

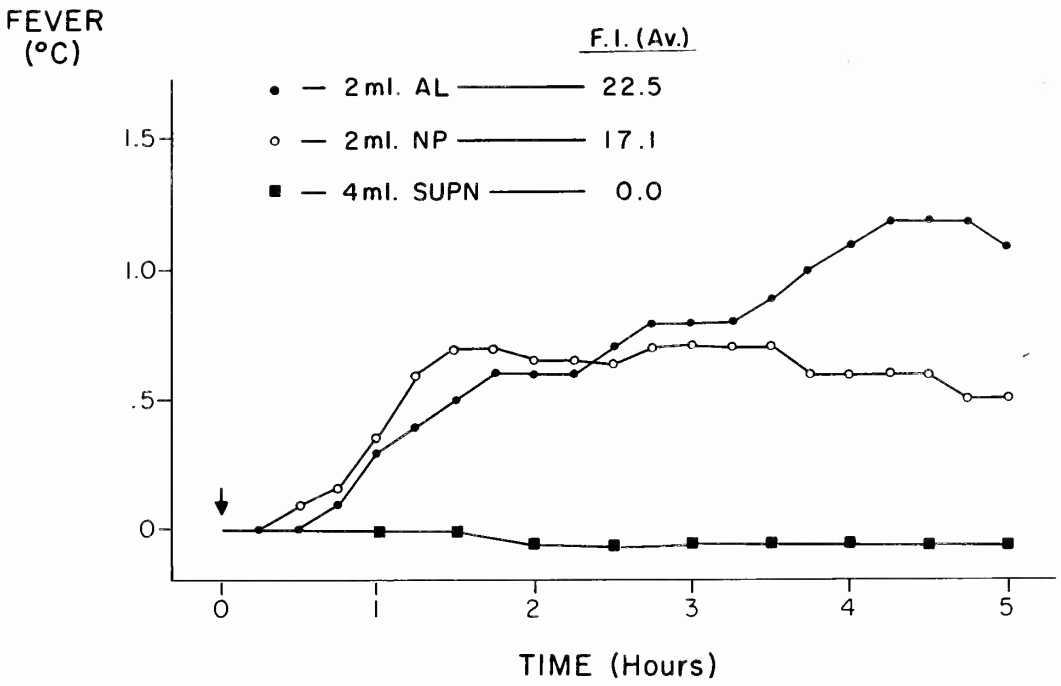


Fig. 5 Mean febrile responses of three vaccinated recipients to pneumococcal autolysate, a solution of isolated pneumococcal nucleoprotein, and the protein-free supernate. All injected materials were non-pyrogenic in normal controls.

Note that, like autolysate, the nucleoprotein solution elicited a febrile response in the vaccinated animals, while the supernate, free of protein, was non-pyrogenic.

Further Studies in the Pyrogenicity of Pneumococcal Nucleoprotein

Two vaccinated animals were injected on separate occasions with the following materials:

(a) 2 ml. autolysate

(b) nucleoprotein derived from autolysate by precipitation with acetic adid and resuspended in saline to the original volume of autolysate. This solution contained 1500 γ. protein per ml. Two ml. were injected.

(c) the acetic acid supernate from a different preparation which contained 500 γ. protein per ml. Three ml. were injected.

(d) another acetic acid supernate, with 440 γ. protein per ml. Two ml. were injected. The results are shown in Fig. 6.

Note that all inocula containing nucleoprotein elicited a febrile response in the test animals and that the height and duration of the fevers varied directly with the quantity of protein injected. This finding supplies further evidence that the pyrogenic substance in the pneumococcal autolysate is the nucleoprotein.

Tolerance to Intravenous Autolysate

Hall and Atkins (11) observed that daily intravenous injections

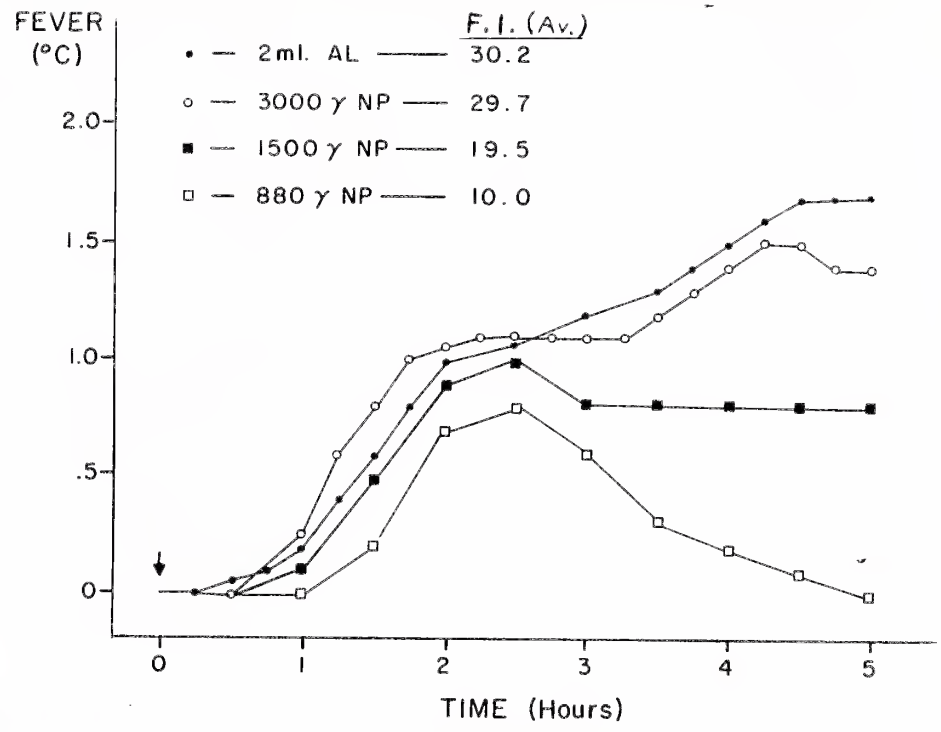


Fig. 6 Mean febrile responses of two vaccinated recipients to autolysate and to inocula containing 3000 γ , 1500 γ and 880 γ nucleoprotein. All injected materials were non-pyrogenic in normal controls.

of OT in BCG-infected rabbits resulted in a rapid tolerance to its pyrogenic effect together with the development of a negative skin test. The correlative loss of dermal and febrile reactivity suggested that the mechanism of tolerance was specific desensitization.

It was considered pertinent to attempt to induce tolerance in animals reactive to pneumococcal autolysate. Accordingly one group of vaccinated animals was given three daily injections of two ml. autolysate. Their daily fevers are recorded in Fig. 7. Note the almost complete loss of febrile response after the first intravenous injection.

Another group of vaccinated animals was skin tested, injected several days later with 2 ml. of autolysate, and skin tests performed again at intervals varying from the same day to four days after injection. Results are shown in Table II.

Note the absence of skin reactivity on the day following autolysate injection in five out of the six animals tested. Note also that in the two or three day group skin reactivity is present but reduced from previous levels, while at four or five days it is essentially undiminished from previous levels.

These findings indicate that the intravenous injection of autolysate induces the loss of both dermal and febrile reactivity in vaccinated animals. It appears that within a few days the skin reactivity returns to previous levels.

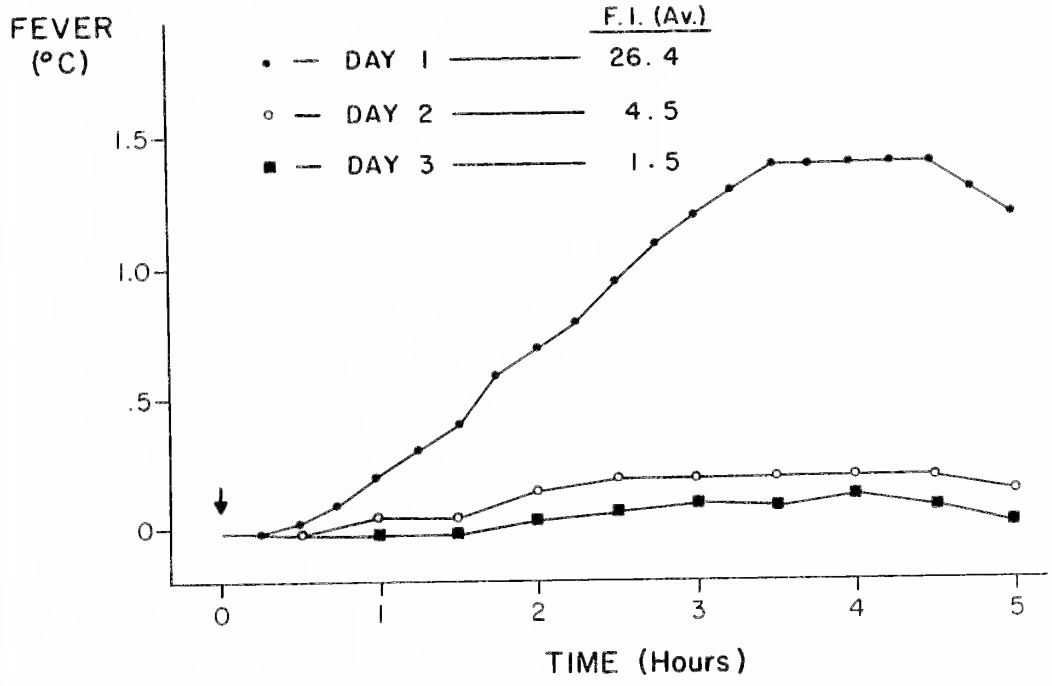


Fig. 7 Mean febrile responses of six vaccinated recipients to three daily injections of autolysate.

TABLE II

SKIN REACTIONS OF VACCINATED RABBITS BEFORE AND AFTER INTRAVENOUS INJECTION OF AUTOLYSATE. ANIMALS ARE DIVIDED INTO THREE GROUPS ACCORDING TO THE INTERVAL BETWEEN THE INJECTION OF AUTOLYSATE AND THE READING OF THE SKIN TEST PERFORMED AFTER INJECTION. ALL READINGS WERE MADE AT 24 HOURS

	One day			2-3 days			4-5 days	
Animal	Skin reaction before injection	Skin reaction after injection	Animal	Skin reaction before injection	Skin reaction after injection	Animal	Skin reaction before injection	Skin reaction after injection
A-1	15x15	neg	B-1	20x20	10x10	C-1	30x30	28x28
A-2	20x15	neg	B-2	25x20	20x15	C-2	35x30	35x30
A-3	24x20	neg	B-3	28x25	25x20	C-3	25x25	25x25
A-4	20x20	neg	B-4	25x25	25x25	C-4	20x15	20x15
A-5	15x10	neg						
A-6	24x20	23x20						

Return of Febrile Response to Autolysate in Animals Made Tolerant

Fig. 8 shows the return of febrile reactivity to autolysate in two animals rendered tolerant to autolysate by two daily injections. The level of reactivity prior to tolerance is shown for comparison. It is seen that in two weeks the febrile response has returned to the previous level.

This finding parallels that made by Moses and Atkins in tuberculin-sensitive rabbits made tolerant to OT (20). The present system demonstrates in addition a correlative recovery of skin reactivity (above).

Loss of Febrile Response to Autolysate

Moses and Atkins observed that the pyrogenic effect of OT in rabbits vaccinated with BCG was maximal three weeks after vaccination, began to wane at five weeks and was considerably reduced by 12 weeks although fevers could still be elicited at this time (20). They attributed this decline in reactivity to the natural loss of hypersensitivity to tuberculin.

Three rabbits vaccinated with heat-killed pneumococci were tested for febrile reactivity to 2 ml. of pneumococcal autolysate at periods of 2 1/2 and 8 weeks after vaccination. Another group of vaccinated animals was tested two and twelve weeks after vaccination. Results are shown in Figs. 9 and 10.

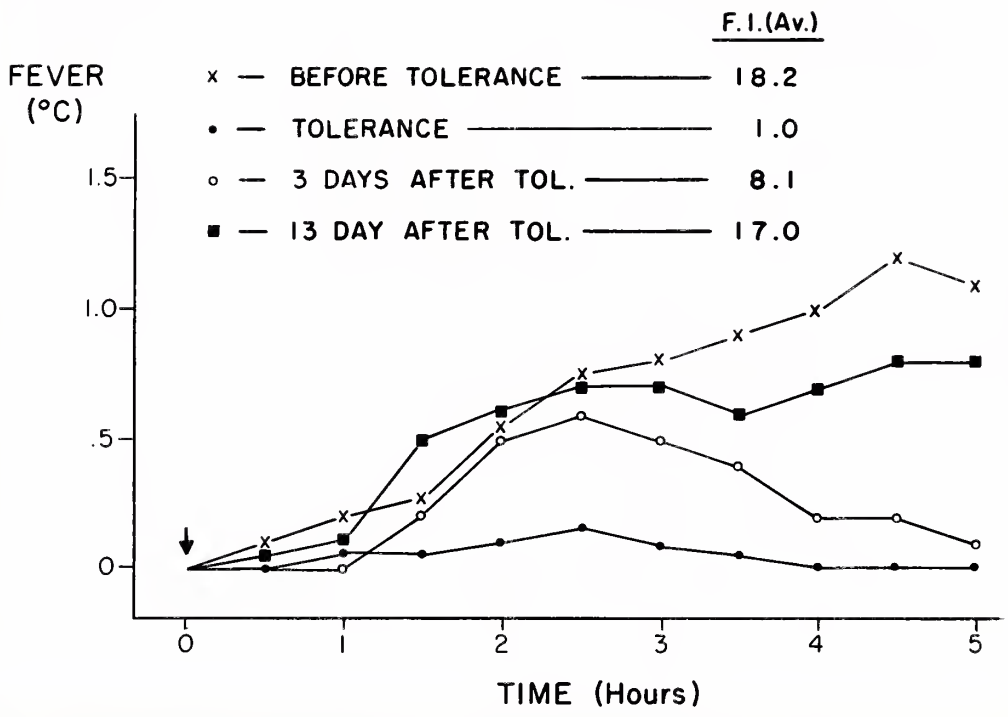


Fig. 8 Mean febrile responses of two vaccinated animals made tolerant to autolysate and tested three and 13 days after tolerance. Average pre-tolerance response is shown for comparison.

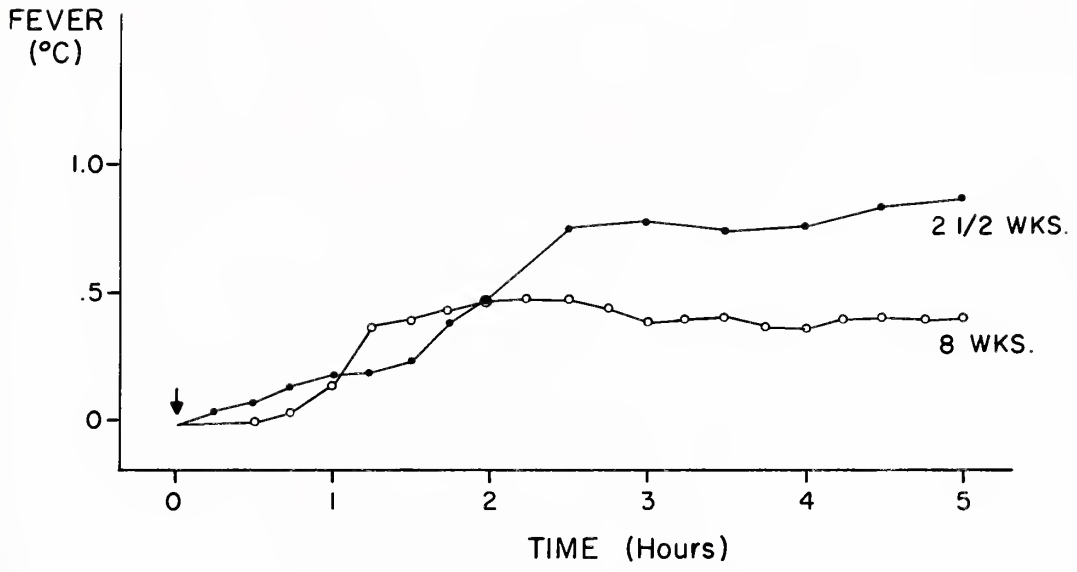


Fig. 9 Mean fevers of three vaccinated recipients injected with autolysate 2 1/2 and eight weeks after vaccination.

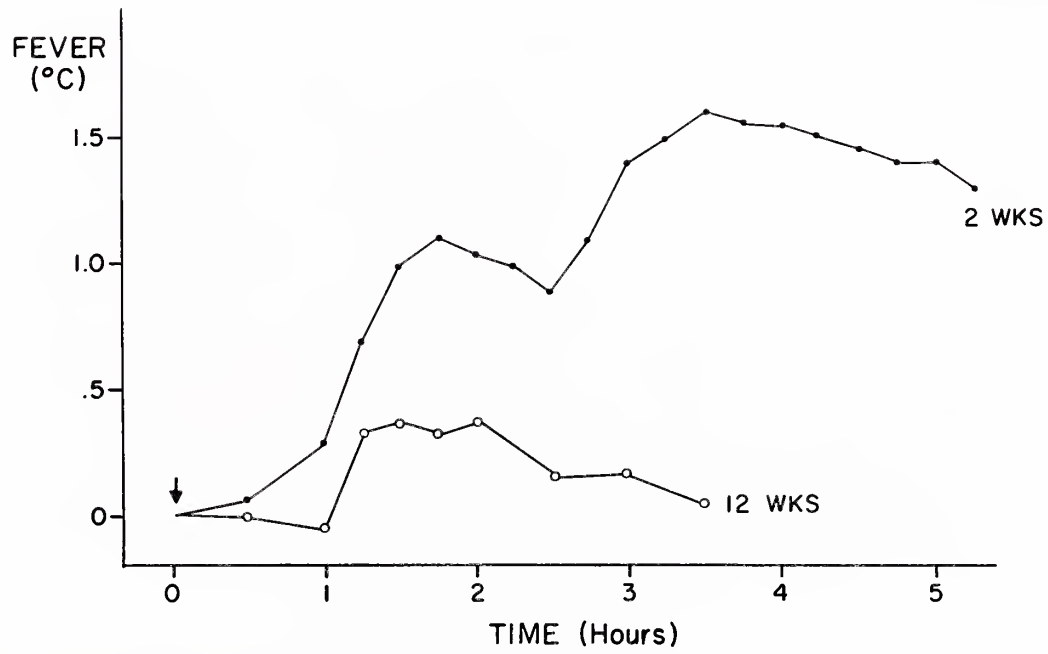


Fig. 10 Mean fevers of three vaccinated recipients injected with autolysate two and 12 weeks after vaccination.

Note the decline in reactivity at eight weeks and the even more diminished response at 12 weeks.

Pyrogenicity of Pneumococcal Serum Filtrates in Vaccinated Rabbits

Atkins noted that in rabbits intensively exposed to Staphylococcus aureus, fevers were induced by the intravenous injection of lysed staphylococci as well as of cell-free filtrates of staphylococcal cultures grown in pyrogen-free media (to be published.)

An attempt was undertaken to parallel this finding in the pneumococcal system. Pneumococci were grown in rabbit serum (pyrogen-free) and the 18 hour cultures were filtered to remove the cells. The cell-free filtrate was injected in varying quantities into three normal and three vaccinated animals. Results are shown in Fig. 11. Note the pyrogenicity of the serum filtrate in the vaccinated recipients.

Fig. 12 compares the reactivity of two vaccinated rabbits to 10 ml. serum filtrate and to 2 ml. autolysate. Note the close similarity of the fevers induced. This finding suggests that the serum filtrate contains small quantities of pneumococcal protein, perhaps as a result of the spontaneous lysis of pneumococci during the growth phase or during handling of the culture.

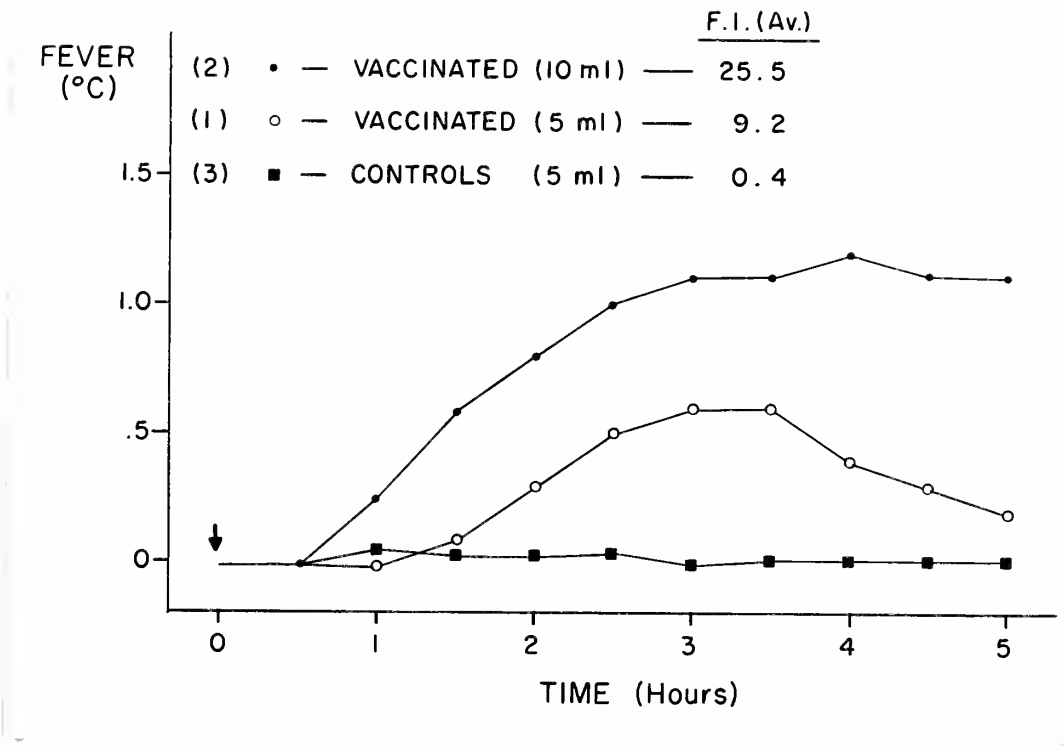


Fig. 11 Mean fevers of vaccinated and normal recipients injected with pneumococcal serum filtrate. Figures to the left of key represent number of animals in each group; volumes in parentheses indicate the amount of material injected.

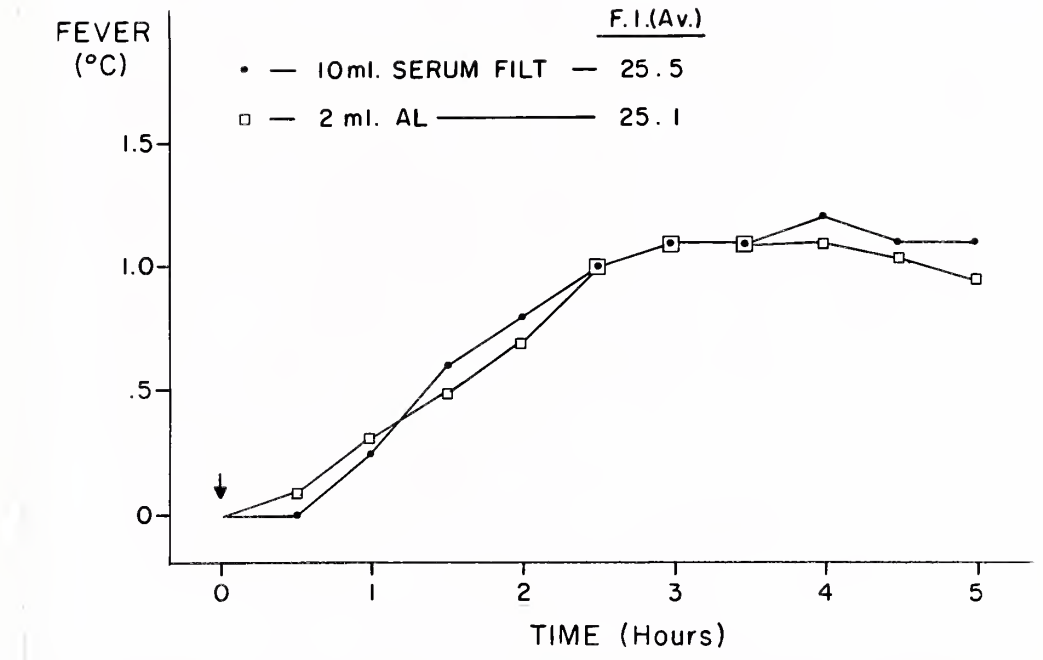


Fig. 12 Mean febrile responses of two vaccinated recipients to 10 ml. serum filtrate and 2 ml. pneumococcal autolysate.

Pyrogenicity of Pneumococcal Autolysate in Animals Vaccinated with Freund's Adjuvant and Saline

All experiments in the pyrogenicity of pneumococcus and pneumococcal derivatives were conducted among a small colony of rabbits. These animals were housed in a single air-conditioned room. During the course of experimentation a series of staphylococcal infections struck the colony, killing some animals and in one degree or another exposing all to the organism. Shortly after a particularly serious epizootic of this nature, it was decided to undertake a control study of the autolysate fevers that had been observed in rabbits vaccinated with heat-killed pneumococci in Freund's adjuvant. It was considered that Freund's adjuvant in itself might play a role in the development of sensitivity to pneumococcus.

Nine animals were given two intradermal vaccinations of a 50/50 mixture of Freund's complete adjuvant and physiologic saline. The animals were skin tested with pneumococcal autolysate at ten days, and at 2 - 3 weeks were tested for febrile reactivity to 2 ml. of the autolysate (AL). Because of the findings, they were subsequently injected with 4 ml. of a filtered preparation of lysed staphylococci Giorgio (mickled staphylococcal filtrate, SF; see Methods). Responses are summarised in Table III.

TABLE III

DERMAL AND FEBRILE REACTIONS OF NINE ANIMALS VACCINATED WITH FREUND'S ADJUVANT AND SALINE. BLANKS ARE INSERTED IN CASES WHERE THE ANIMAL WAS NOT TESTED

Animal	Skin reaction	AL fever	SF fever
R-1	neg	0.0° C.	0.0° C.
R-2	--	0.2	--
R-3	neg	0.2	--
R-4	neg	0.4	0.9
R-5	neg	0.4	2.2
R-6	neg	0.6	1.3
R-7	neg	1.0	1.5
R-8	neg	1.0	--
R-9	neg	1.2	1.5

Note that while skin reactions were negative in all the animals tested, there is present a spectrum of AL fevers ranging from 0.0° to 1.2° C. Note also that the responses of the animals injected with SF roughly parallel the responses to AL. The findings suggest: (a) that these "control" animals have become reactive to lysed staphylococci by exposure to the organism, and (b) that this exposure has conferred on the animals a cross-reactivity to pneumococcal autolysate.

Pyrogenicity of Pneumococcal Autolysate in Rabbits Reactive to Mickled Staphylococcal Filtrate.

After the epizootic mentioned above, 19 animals having had no vaccinations of any type were tested for febrile reactivity to 4 ml. SF and to 2 ml. AL on separate occasions. Twelve of these animals proved to be reactive to both materials. Five were unreactive to both. Average responses to SF and to AL are shown in Fig. 13 and 14 respectively. Note that both responses have a 30 to 60 minute latent period, similar to that of AL fever in pneumococcus vaccinated rabbits.

The remaining two animals reacted to SF with high fever but were only slightly responsive to AL. The SF and AL fever indices of all 14 reactive animals are compared in Fig. 15. Note the lack

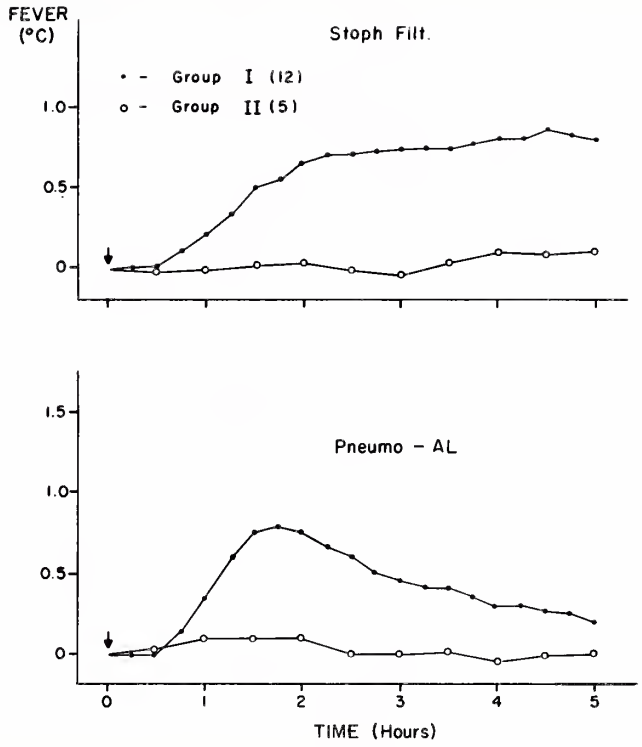


Fig. 13 Mean febrile responses of non-vaccinated rabbits to 4 ml. mickled staphylococcal filtrate. Animals are divided into two groups according to reactivity.

Fig. 14 Mean febrile responses of the same two groups to 2 ml. pneumococcal autolysate.

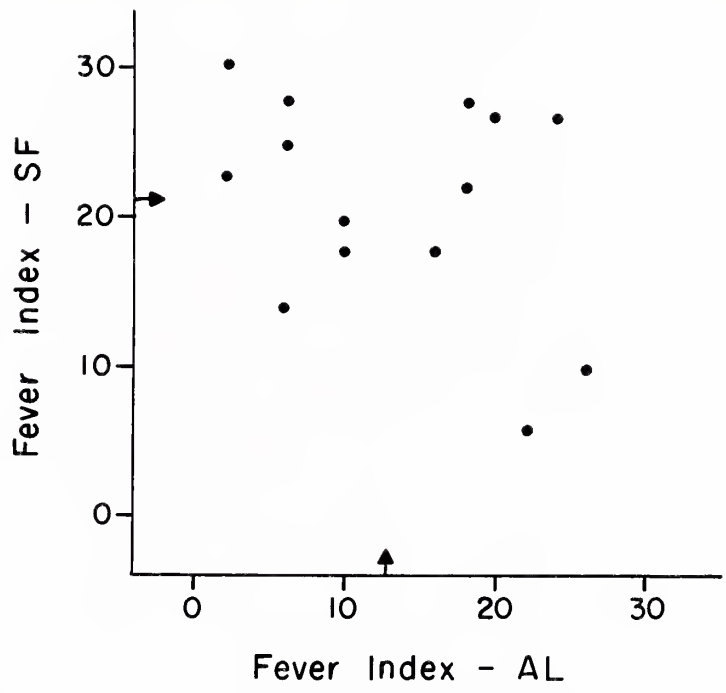


Fig. 15 Correlation of febrile responses to SF and to AL in 14 non-vaccinated rabbits. Arrows indicate the mean fever index.

of correlation between the magnitude of the response to SF and that to AL.

It is seen that, in the absence of prior manipulation, the majority of the animals tested demonstrate a characteristic febrile response to staphylococcus which is closely coincident with a spontaneous reactivity to pneumococcus. This finding supports the suggestion that exposure to staphylococcus confers reactivity to both staphylococcus and pneumococcus.

Dermal Sensitivity to AL in Rabbits Reactive to SF

Skin tests with pneumococcal autolysate were performed on ten non-vaccinated rabbits. In five of these animals both SF and AL fevers had been demonstrated. The other five had not been responsive to either material. The results are summarized in Table IV. Shown for comparison are the skin responses of rabbits vaccinated with pneumococcus.

It is seen that with one exception skin reactivity is not found in the vaccinated animals. Apparently, animals in which exposure to staphylococcus is associated with febrile reactivity to autolysate do not generally develop dermal reactivity as well.

Correlation of AL Fevers in Vaccinated and Non-Vaccinated Rabbits

Fig. 16 superimposes the mean febrile responses to 2 ml.

TABLE IV

AUTOLYSATE SKIN REACTIONS OF TEN NON-VACCINATED AND 41 PNEUMOCOCCUS VACCINATED RABBITS

Group	Number tested	Skin reactions
Non-vaccinated, had both SF fever (av. ht. 1.3° C) and AL fever (av. ht. 0.9° C)	5	1 pos (20 mm) 4 neg
Non-vaccinated, not responsive to SF or AL	5	5 neg
Vaccinated with heat-killed pneumococci	41	36 pos 5 neg

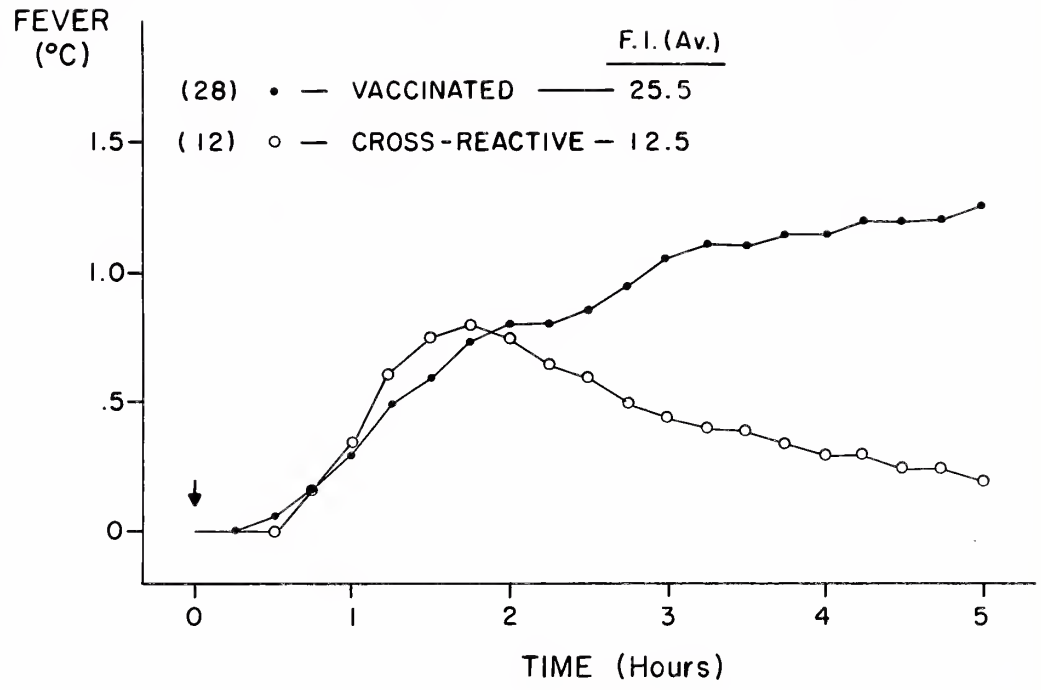


Fig. 16 A comparison of AL fevers in rabbits vaccinated with pneumococcus and in non-vaccinated animals "cross-reactive" with staphylococcus. Ratio of fever indices is two to one.

AL of 28 rabbits vaccinated with pneumococcus and 12 non-vaccinated animals "cross-reactive" with staphylococcus. Note the similar latencies of both fevers. Observe also that the ratio of fever indices of vaccinated to non-vaccinated animals is two to one.

Cross-Tolerance in Vaccinated Rabbits

On the basis of findings suggestive of cross-reactivity between staphylococcus and pneumococcus in non-vaccinated rabbits, it was decided to test rabbits that had been vaccinated with pneumococcus for their reactivity to AL and to SF, attempt to establish tolerance to the pyrogenic effect of SF, and then test for cross-tolerance to AL. It was considered that a negative response to AL after "desensitization" with SF would supply further evidence of cross-reaction between the two organisms.

Accordingly, six vaccinated rabbits were injected as follows: day one - 2 ml. AL; day two - no injection; day three - 6 ml. SF; day four - 6 ml. SF; day five - 2 ml. AL. In a control study, six animals received 2 ml. AL on days one and five, with no injections in the interim. Results are shown in Figs. 17 and 18.

Noteworthy in the test group are: (a) the large response to AL on day one and to SF on day three; (b) the appearance of tolerance to SF on day four; (c) the markedly diminished response to AL on

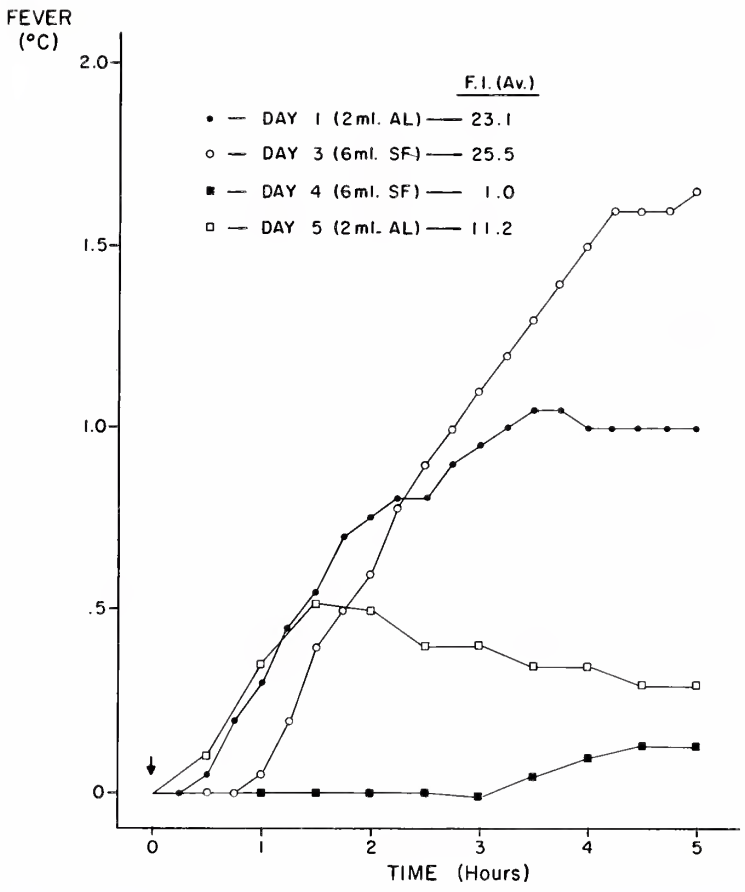


Fig. 17 Mean febrile responses of six vaccinated recipients to AL and SF. Schedule of injections is given in the key.

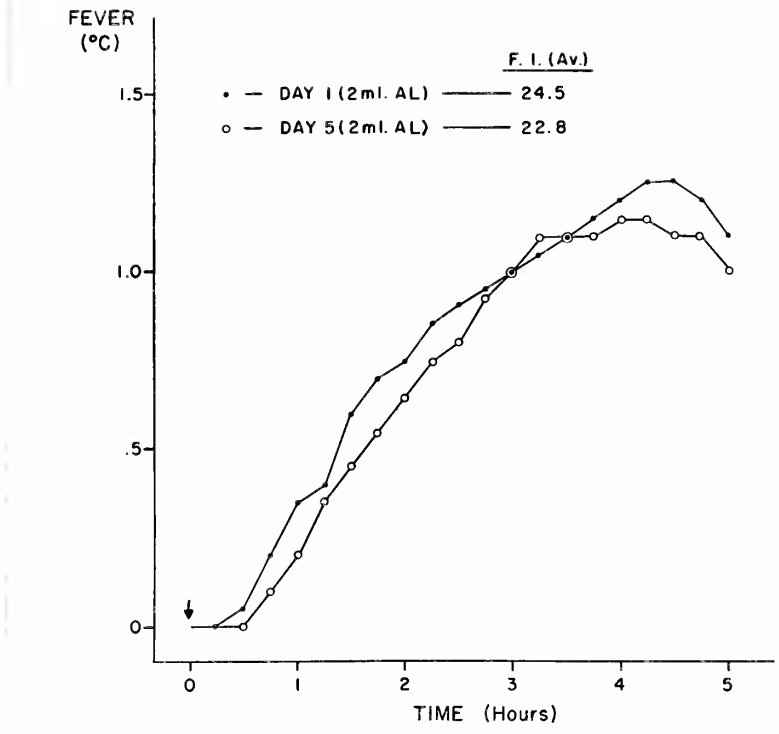


Fig. 18 Mean febrile responses of six vaccinated controls to two injections of AL. Schedule is given in the key.

day five. Observe that in the control group, reactivity to AL on day five does not differ notably from that on day one.

These findings suggest that vaccinated animals made tolerant to SF become cross-tolerant to AL.

Individual Variation in Cross-Tolerant Animals

Fig. 19 shows the individual responses of the six SF-tolerant recipients to AL on day five. Average AL responses on day one and day five are shown for comparison. Note that at some points individual fevers on day five exceed the average response on day one. Despite this variation, the ratio of the mean fever indices is approximately two to one.

Skin Reactivity to AL in Vaccinated Animals Made Tolerant to SF

Five vaccinated animals, all skin-sensitive to AL, were injected as follows: day one - 2 ml. AL; day three and day four - 6 ml. SF. After tolerant responses were obtained on day four, AL skin tests were performed. In a control study, seven skin-reactive animals received 2 ml. AL on day one and were skin tested again on day four. Results are summarized in Table V. Skin reactions were read at 24 hours.

It is seen that in both tolerant and control groups there is some loss of skin sensitivity. Because of the limited number of animals tested, the findings must be considered inconclusive.

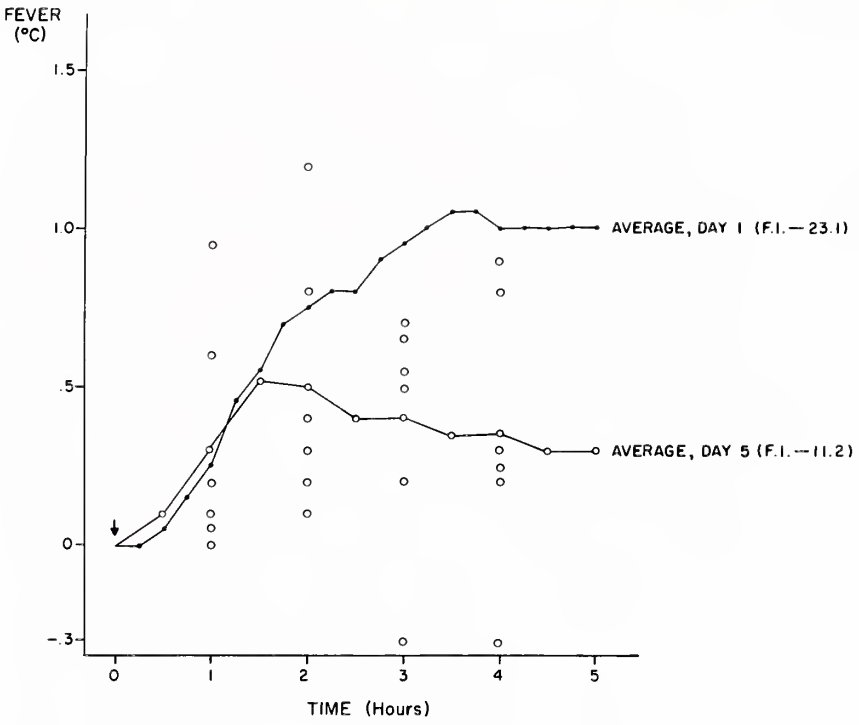


Fig. 19 Individual responses of six SF-tolerant recipients to AL on day five. Mean responses on day one and day five are shown for comparison.

TABLE V

SKIN REACTIONS OF FIVE VACCINATED ANIMALS MADE TOLERANT TO SF AND SEVEN VACCINATED CONTROLS. PRIOR TO INTRAVENOUS INJECTION ALL ANIMALS HAD BEEN SKIN-TEST POSITIVE

Group	Skin reactions
Tolerant	3 pos
	2 neg
Controls	5 pos
	2 neg

DISCUSSION

There has been some question as to the type of fever that develops in normal rabbits after the intravenous injection of pneumococci. The present study indicates that the febrile response to the live and heat-killed organisms is similar in its 90 minute latency to fevers induced by other gram-positive bacteria. That the integrity of the cell body is essential to its pyrogenicity in normal animals is demonstrated by the failure of cell-free autolysates to elicit fever. This finding is consistent with the hypothesis that the febrile response to gram-positive organisms is related to the process of phagocytosis by circulating leucocytes. Since killing with heat reduces the pyrogenicity of pneumococci, factors other than particulate-ness may be involved, such as electrical charge, configurations at the cell surface, etc. Again, it is possible that the more transient fevers elicited by heat-killed cells reflect clearance of these organisms from the blood stream at a time when live pneumococci would be expected to be multiplying and, presumably, inducing progressively greater phagocytic response.

The conditions and characteristics of febrile reactivity to pneumococcal autolysate in rabbits vaccinated with pneumococci suggest strongly that this responsiveness is a manifestation of a specific acquired hypersensitivity. Prior to the occurrence of

staphylococcal infections in the colony of test animals, fevers produced by the intravenous injection of pneumococcal autolysate were noted exclusively in animals that had been specifically vaccinated and were almost always associated with positive skin tests. In latency and appearance the febrile response to autolysate closely resembles the fevers seen in other systems of hypersensitivity, particularly the tuberculin system (11). The development of tolerance to a second daily injection of autolysate, occurring as it does in conjunction with the loss of skin reactivity, is strongly suggestive of specific desensitization. The rapidity with which febrile reactivity returns after tolerance, and the correlative return of dermal reactivity, argue further for hypersensitivity. The gradual spontaneous loss of responsiveness to autolysate in vaccinated animals resembles the decline of tuberculin reactivity in BCG-infected rabbits (20) and suggests a natural "wearing off" of the hypersensitive state.

Since neither the febrile nor the dermal response of vaccinated rabbits to pneumococcal nucleoprotein were correlated with circulating precipitins, both responses appear to be manifestations of delayed (cellular), rather than immediate (humoral) allergy. It must be borne in mind, however, that in one model immunologic system reactivity has been shown to depend on serum antibody that is non-precipitating in type (7, 8). The participation of such a factor in the autolysate

system, seems doubtful, in view of the characteristically delayed skin reaction, but experiments of the passive transfer type would be required to rule out this possibility conclusively. On the basis of present evidence, one would expect in such an experiment that reactivity to autolysate would be conveyed from sensitive to normal animals by transfer of leucocytes, as in the tuberculin system (15), rather than by serum.

The spontaneous appearance in non-vaccinated animals of autolysate fevers similar to those observed in pneumococcus-vaccinated rabbits was an unexpected finding. It raises the possibility that cellular hypersensitivity may play a significant role in the pathogenesis of naturally acquired fevers caused by gram-positive bacteria. The animals in the present study appear to have developed reactivity to pneumococcus secondarily to intensive exposure to staphylococcus. The evidence for this cross-reactivity is as follows:

(1) Autolysate fevers were not observed in "normal" rabbits prior to the occurrence of a series of epizootics in the laboratory colony due to S. aureus.

(2) In the majority of animals tested subsequently, there proved to be a close coincidence of febrile reactivity to lysed staphylococci Giorgio and to pneumococcal

autolysate. In latency and appearance these reactions to staphylococcal products resembled the autolysate fevers of pneumococcus-vaccinated animals.

(3) Vaccinated animals demonstrated reactivity to the staphylococcal preparation as well as to pneumococcal autolysate. After a characteristic tolerant response to the staph. filtrate was induced by repeated injection, the animals proved to be partially tolerant to the autolysate as well.

A comparison of autolysate fevers in vaccinated and in non-vaccinated staph.-exposed animals shows that the mean fever indices of the two responses are in the ratio of two to one. When autolysate fevers in vaccinated rabbits are compared before and after staph. tolerance, it is seen that the mean fever indices are also in the ratio of two to one. It appears as if the amount of autolysate reactivity associated with staph. reactivity were a constant which could be subtracted by rendering an animal tolerant to staphylococcal lysate. This suggests an antigenic overlap between the two organisms. Possibly animals vaccinated with pneumococcus develop a number of antibodies of which a fraction are also elicited by exposure to staphylococci. Since it is the protein component of the pneumococcus that contains the pyrogen,

it may be that the two gram-positive organisms share certain proteins in common.

One feature of the cross-reactive system which appears inconsistent with other findings is the absence of skin reactivity to pneumococcal autolysate in the staph.-exposed rabbits. The relationship of factors governing dermal and febrile responsiveness is not well understood. In other systems of hypersensitivity the size of the skin reaction in rabbits has been found to bear little relation to the magnitude of the fever, and in some instances one response may occur without the other. This has already been noted here in rabbits vaccinated with pneumococcus, and it has also been reported in the tuberculin system (11).

The findings reported in this paper suggest that the clinical response to pneumococcal infection may to some extent be influenced by prior sensitization of the host. It is conceivable that previous experience with the specific organism or with a cross-reactive strain of staphylococcus has the effect of intensifying the inflammatory response in the manner seen in secondary infection with the tubercle bacillus. The outpouring of leucocyte-rich exudates at the infected site would allow for an extensive interaction of bacterial antigen with cellular antibody, with the subsequent release of large quantities of

endogenous pyrogen into the circulation. An immunologic reaction of this type may account in part for the high and prolonged fevers that are commonly seen in clinical pneumococcal disease.

SUMMARY

The present study indicates that normal rabbits respond to intravenous injections of live and heat-killed pneumococci type I with fevers of one to two hour latency. The integrity of the cell body appears to be essential to its pyrogenicity, since cell autolysates were not pyrogenic for normal animals.

On the other hand, autolysates of pneumococci induced fevers in animals that had previously been vaccinated with heat-killed organisms. The capacity to respond to autolysate with fevers was regularly associated with delayed cutaneous reactions to this material. Both these reactions appeared to be unrelated to the presence of circulating antibody. One intravenous injection of autolysate produced tolerance to its pyrogenic effect together with the loss of skin reactivity. After a short rest period both dermal and febrile reactivity returned, but were gradually lost after several weeks. On the basis of these findings it is postulated that the fever elicited by pneumococcal autolysate in vaccinated animals is a manifestation of delayed hypersensitivity.

Evidence has been presented that intensive exposure to Staphylococcus aureus induces in non-vaccinated animals a cross-reactivity to pneumococcal autolysate. In such exposed animals there was found to be a close coincidence of febrile reactivity to staphylococcal and to pneumococcal lysates. Furthermore, rabbits vaccinated

with pneumococcus and given repeated injections of lysed staphylococci regularly developed partial tolerance to pneumococcal autolysate. It is suggested that there is an area of antigenic overlap between the two gram-positive organisms, perhaps among the somatic proteins.

The possible clinical significance of these findings is briefly discussed.

BIBLIOGRAPHY

1. Atkins, E. Pathogenesis of fever. Physiol. Rev., 40:580, 1960.

2. Atkins, E. and Freedman, L. Studies on the mechanism of fever following intravenous inoculation of staphylococci. J. Clin. Invest., 39:969, 1960.

3. Atkins, E. and Huang, W. C. Studies on the pathogenesis of fever with influenzal viruses. I. The appearance of an endogenous pyrogen in the blood following intravenous injection of virus. J. Exp. Med., 107:383, 1958.

4. Atkins, E. and Wood, W. B., Jr. Studies on the pathogenesis of fever. II. Identification of an endogenous pyrogen in the blood stream following the injection of typhoid vaccine. J. Exp. Med., 102:499, 1955.

5. Bennett, I. L., Jr. Studies on the pathogenesis of fever. V. The fever accompanying pneumococcal infection in the rabbit. Bull. Johns Hopkins Hosp., 98:216, 1956.

6. Eisler, R., Moeller, H. C. and Grossman, M. I. Report on febrile response of rabbits to intravenous injection of colloidal substances. Army Med. Nutrit. Lab. Rep., Denver 9937 TU, Rept. No. 167, June 1955.

7. Farr, R. S. The febrile response upon injection of bovine albumin into previously sensitized rabbits. J. Clin. Invest., 37:894, 1958.

8. Grey, H. M., Briggs, W. and Farr, R. S. Passive transfer of sensitivity to antigen-induced fever. J. Clin. Invest., 40:703, 1961.

9. Goodner, K. Experimental intradermal pneumococcus infection in rabbits. J. Exp. Med., 48:1, 1928.

10. Hagebusch, O. E., Robben, F. J., Fleisher, M. S. and Jones, L. Serum sickness in rabbits. III. Reactions of body temperature and leucocytic curves. J. Immunol., 22:373, 1932.

11. Hall, C. H., Jr. and Atkins, E. Studies on tuberculin fever. I. The mechanism of fever in tuberculin hypersensitivity. J. Exp. Med., 109:339, 1959.

12. Harley, D. A study of pneumococcal allergy, anaphylaxis and immunity. J. Path. & Bact., 41:491, 1935.

13. Harley, D. A study of pneumococcal allergy and immunity. J. Path & Bact., 45:257, 1937.

14. Hort, E. C. and Penfold, W. J. Microorganisms and their relation to fever. J. Hyg., 12:361, 1912.

15. Johanovsky, J. Demonstration of endogenous pyrogen in serum during systemic tuberculin reaction in rabbits. Nature, London, 183:693, 1959.

16. Julianelle, L. Reactions of rabbits to intracutaneous injections of pneumococci and their products. I. The antibody response. J. Exp. Med., 51:441, 1930.

17. Julianelle, L. Reactions of rabbits to intracutaneous injections of pneumococci and their products. VI. Hypersensitiveness to pneumococci and their products. J. Exp. Med., 51:643, 1930.

18. King, M. K. and Wood, W. B., Jr. Studies on the pathogenesis of fever. V. The relation of circulating endogenous pyrogen to the fever of acute bacterial infections. J. Exp. Med., 107:305, 1958.

19. Lowry, O. H., Rosebrough, N. J., et cal. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193:265, 1951.

20. Moses, J. M. and Atkins, E. Studies on tuberculin fever. II. Observations on the role of endogenous pyrogen in tolerance. J. Exp. Med., 114:939, 1961.

21. Mudd, S. and Wiener, M. The antigenic structure of hemolytic streptococci of Lancefield group A. XI. Relationships of the nucleoproteins of some species of streptococci and pneumococci. J. Immunol., 45:21, 1942.

22. Ouchterlony, O. Diffusion-in-gel methods for immunologic analysis. Prog. Allergy., 5:26, 1958.

23. Probey, T. F. and Pittman, M. Pyrogenicity of bacterial contaminants found in biologic products. J. Bact., 50:397, 1945.

24. Stetson, C. A., Jr. Studies on the mechanism of the Shwartzman phenomenon. Similarities between reactions of endotoxins and certain reactions of bacterial allergy. J. Exp. Med., 101:421, 1955.

25. Uhr, J. W. and Brandriss, M. W. Delayed hypersensitivity. IV. Systemic reactivity of guinea pigs sensitized to protein antigens. J. Exp. Med., 108:905, 1958.

26. Uhr, J. W. and Pappenheimer, A. M. Delayed hypersensitivity. III. Specific desensitization of guinea pigs sensitized to protein antigens. J. Exp. Med., 108:891, 1958.

27. White, B., Robinson, E. S. and Barnes, L. A. The Biology of pneumococcus: The bacteriological, biochemical and immunological characters and activities of diplococcus pneumoniae. New York, Commonwealth Fund, 1938.

28. Wright, H. D. Experimental pneumococcal septicaemia and antipneumococcal immunity. J. Path. & Bact., 30:185, 1927.

3 9002 00753 3988

MUDD

Date Due

All books are subject to recall after two weeks.

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

_____

_____

NAME AND ADDRESS DATE